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THE EFFECTS OF ACUTE ALTERATIONS IN HEMODYNAMICS,
OXYGEN AVAILABILITY AND ACID-BASE BALANCE ON
THE PERMEABILITY OF THE GASTRIC MUCOSA

ANNUAL PROGRESS REPORT
(FOR THE PERIOD 1 OCT. 77 TO 30 SEPT. 78)

DATE OF REPORT May 24, 1978

BY

WALLACE P. RITCHIE, JR., M.D.

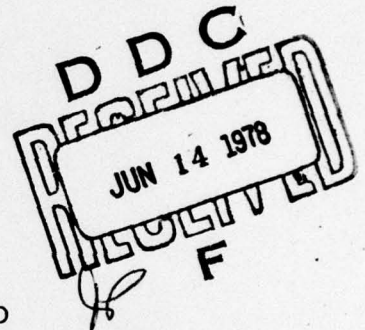
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Using a previously described model for acute gastric mucosal ulcerogenesis (Gastroent. 68:699, 1975), studies carried out in this laboratory during the period covered by the progress report indicate that acute gastric mucosal ulcerogenesis may be a consequence of uncompensated tissue acidosis; that the topical application of 16,16 DM PGE ₂ significantly ameliorates bile acid-ischemia induced acute mucosal lesion formation; and that intravenous metiamide is cyto-protective under the same circumstances, perhaps as a consequence of its ability to decrease the apparent permeability of the gastric mucosa to cations.		

PROGRESS REPORT: CONTRACT DAMD17-74-C-4014

I. TITLE OF RESEARCH CONTRACT:

The Effects of Acute Alterations in Hemodynamics, Oxygen Availability, and Acid-Base Balance on the Permeability of the Gastric Mucosa

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IV. PROGRESS REPORT

(1) Use of Radiolabeled Microspheres to Measure Gastric Mucosal Blood Flow. A principle goal of the current year has been to establish in our laboratory the capacity to estimate gastric mucosal nutrient blood flow using the radiolabeled microsphere technique originally described and validated by Archibald et al (Gastroent. 69:630, 1975). Briefly, the technique consists of injecting a pre-mixed solution of approximately 100,000 to 500,000 microspheres of uniform diameter, tagged with γ emitters of different energy spectra (^{51}Cr , ^{141}Ce , ^{85}Sr), into the left ventricle. A reference sample is drawn at 10ml/min from the distal aorta for 80 seconds, beginning 10 seconds before injection. At the conclusion of the experiment the mucosa is sharply separated from the underlying muscular layers and dried at 60 C. The blood and tissue specimens are then dissolved in hot concentrated nitric acid to produce homogenous isotope solutions and are counted in a gamma counting system. Flow is calculated by comparing the activity of each isotope in the tissue with that in the reference sample of plasma as

$$\text{Flow (ml/Gm-min)} = \frac{\text{DPM mucosa (Gm)}}{\text{DPM/ml-min reference}}$$

Because the studies of Archibald et al indicate that submucosal shunting is minimal, even following damage to the gastric mucosa, this parameter has not been assessed.

In a series of preliminary studies, we have been able to confirm the observations of others with respect to the following: (1) the aminopyrine clearance technique for estimating mucosal blood flow underestimates microsphere flow by approximately 1/5 (1.3+0.3ml/min vs 6.2+1.2ml/min); (2) this discrepancy is reduced to approximately 1/3 during histamine stimulation; (3) the topical application of low concentrations of bile acid to the gastric mucosa increases

flow calculated either by the aminopyrine clearance technique (to 2.0 ± 0.3 ml/min) or by the microsphere technique (to $9.4 \pm 1/5$ ml/min); (4) under both circumstances, intraarterial infusion of low doses of vasopressin reduces flow in bile acid treated mucosa. We now feel sufficiently confident with the use of microspheres to employ this methodology on a routine basis.

(2) Differences in A-V Acid Base Balance During Acute Gastric Mucosal Ulcerogenesis. The combination of topical acid, topical bile acid, and nutrient mucosal ischemia is acutely ulcerogenic in the proximal stomach. The possibility that lesion formation under these circumstances is a consequence of inadequate tissue buffering of "back-diffused" intramucosal H^+ was examined indirectly by measuring A-V differences in acid-base parameters across vascularized chambered wedges of canine gastric wall. Four groups of animals were studied during 5 sequential 15 minute periods. In each, the splenic artery and vein (which supply the wedge) were cannulated following in-situ splenectomy. The mucosae of Group A dogs (n=4) were exposed, during each period, to topical acid solution alone (ATS); of group B (n=4), to ATS containing 5mM Nataurocholate (TC); of group C (n=4), to ATS during splenic artery infusion with vasopressin (VP=0.01U/Kg-min); of Group D (n=4), to ATS+TC+VP. Indices evaluated= (1) systemic arterial and splenic venous pH, pCO_2 , base deficit (BD) and $[HCO_3^-]$, (2) net H^+ flux (ΔH^+), (3) aminopyrine clearance (AC), a measure of mucosal blood flow, (4) the degree of mucosal damage induced, the lesion index (LI, graded 0-5), and (5) HCO_3^- "output" ($\mu Eq/15min$) by the wedge, calculated as $[HCO_3^-]_V - [HCO_3^-]_A \times AC$. The results/15 minutes ($\pm SEM$):

	ATS	ATS+TC	ATS+VP	ATS+TC+VP
$\Delta H^+ (\mu Eq)$	-16 ± 14	-395 ± 22	-28 ± 18	-375 ± 25
HCO_3^- "output"	29 ± 8	46 ± 6	17 ± 4	$-14 \pm 5^*$
A-V Diff BD	$+1.0 \pm 0.01$	$+0.9 \pm 0.5$	$+1.3 \pm 0.3$	$+5.5 \pm 0.8^*$
A-V Diff pH	0.05 ± 0.01	0.07 ± 0.01	0.12 ± 0.01	$0.21 \pm 0.03^*$
LI	0.2 ± 0.1	0.4 ± 0.4	0.2 ± 0.1	$4.8 \pm 0.3^*$

*Significant difference vs. ATS, ATS+TC, ATS+VP

Systemic arterial pH was not significantly different between groups. Thus, during acute lesion formation, splenic venous HCO_3^- "output" and pH were depressed, while splenic venous BD was increased, suggesting that mucosal ulcerogenesis may be a consequence of uncompensated tissue acidosis.

(3) Influence of Topical 16,16 DM Prostaglandin E_2 on Bile Acid Induced Acute Gastric Mucosal Ulcerogenesis. It has been suggested that prostaglandins may be cyto-protective to the gastric mucosa under a variety of clinically applicable circumstances. The present study was undertaken to examine this possibility in the model outlined above.

Two groups of dogs, each prepared with vascularized chambered ex-vivo wedges of proximal gastric mucosa, were studied during nine sequential 15 minute study periods. Group A (5 dogs) was exposed during periods 1-3, to topical acid test solution (ATS) during concomitant infusion of the splenic artery with 0.9% NaCl; during 4-6, to ATS+2mM sodium taurocholate (TC), again during splenic artery infusion with saline; and, during 7-9, to ATS+TC+ vasopressin (VP=5x10⁻³U/Kg-min. Group B (7 dogs) was studied in a similar fashion with the exception that the mucosa was pre-treated with topical 16,16 DM PGE₂, 5µg/Kg, 20 minutes prior to periods 1, 4, and 7. During each period, the following parameters of gastric mucosal function were evaluated: (1) net flux of H⁺ and Na⁺; (2) the electrical potential difference (PD); (3) the aminopyrine clearance (AC), a semiquantitative index of mucosal blood flow; and (4) the degree of mucosal damage induced, the lesion index (LI) graded on a 0-5 basis by an independent observer using photographs obtained at the end of each period in every dog. The results/15 minutes (+SEM):

	ATS	ATS+PGE ₂	ATS+TC	+PGE ₂	ATS+TC+VP	+PGE ₂
ΔH ⁺ (µEq)	-24±8	-99±13	-160±21	-179±14	-264±24	-212±23
ΔNa ⁺ (µEq)	+62±7	+166±24*	+118±9	+196±18*	+266±16	+171±12*
PD (mV)	-55±2	-58±2	-36±1	-44±2*	-18±1	-30±2*
AC (ml/min)	1.8±0.4	2.0±0.2	1.7±0.2	2.1±0.2	0.8±0.1	1.1±0.2
LI (0-5)	0	0	0	0	4.4±0.4	1.7±0.4*

*Significant difference vs. control

Conclusion: The topical application of 16,16 DM PGE₂ in large doses affords significant protection against bile acid-ischemia induced acute gastric mucosal ulcerogenesis. This protection is not accomplished by alterations in net H⁺ flux or in gastric mucosal blood flow. Rather, the data suggest that cyto-protection may be related to PGE₂ mediated maintenance of normal mucosal sodium homeostasis.

(4) Influence of the H₂ Antagonist, Metiamide, on Bile Acid Induced Acute Gastric Mucosal Ulcerogenesis. The present study was designed to evaluate the effect of metiamide, an H₂ antagonist, on acute gastric mucosal damage produced by the combination of (1) bile acid induced "back-diffusion" of H⁺ and (2) concomitant gastric mucosal nutrient ischemia. Using vascularized, chambered wedges of proximal canine gastric wall, two groups of dogs were studied during 3 sequential periods. Control group A (9 dogs) was subjected to (1) topical acid test solution alone (ATS), (2) topical ATS+2mM taurocholic acid (TC), and (3) ATS+TC+vasopressin (VP), 5x10⁻³U/Kg-min infused into the splenic artery which supplies the wedge. Study group B was similarly treated, except that, in addition, metiamide (MET), 2µM/Kg-hr, was infused intravenously throughout the study. Parameters evaluated during each period included (1) net flux H⁺, Na⁺; (2) aminopyrine clearance (AC), a semiquantitative index of gastric mucosal blood flow; and, (3) the severity of mucosal damage, the lesion index (LI), graded 0-5 by an independent observer using photographs. Results (+SEM) per 15 minutes:

	ATS		ATS+TC		ATS+TC+VP	
	Control	Met	Control	Met	Control	Met
ΔH^+ (uEq)	-21+8	-27+7	-231+21	-124+18*	-314+17	-211+25*
ΔNa^+ (uEq)	+79+5	+68+7	+190+11	+111+8*	+315+19	+209+15*
AC (ml/min)	3.3+0.3	3.6+0.3	4.7+0.3	4.7+0.3	1.7+0.2	1.9+0.1
LI	0	0	0	0	4.4+0.2	2.3+0.3*

*P=or <0.005 vs. control

Conclusions: (1) Metiamide significantly protects against acute mucosal damage induced by bile acids and ischemia. (2) This protection is not mediated by alterations in gastric mucosal blood flow. (3) Rather, metiamide may be protective by virtue of its ability to decrease the apparent permeability of the gastric mucosa to cations.

(5) Tissue Water Distribution in Bile Acid Treated Mucosa. In 8 dogs, chambered wedges of proximal gastric wall were prepared as described above. An in-situ splenectomy was performed to reduce the distribution space of the extracellular water marker employed, ^{14}C -inulin, without compromising the blood supply to the wedge. The superior splenic artery and vein were cannulated. Acid test solution was introduced into the mucosal chamber, and the splenic artery was infused with 0.9% NaCl for 30 minutes. At the end of this time an aliquot of splenic venous blood and a sample of mucosa were obtained for plasma and tissue blanks.

A constant intraarterial infusion of 0.9% NaCl containing sufficient ^{14}C -inulin to produce a specific activity in venous plasma of 20-100 times background was then begun. Venous plasma samples were obtained at 15 minute intervals and counted in a liquid scintillation system to ascertain when steady state conditions existed.

Once these had been achieved, the extracellular, intracellular, and total tissue water were calculated by methods described in detail in the current contract. Briefly, 100-200mg slices of mucosa were weighed and solubilized. A 1ml aliquot was pipeted into 9ml of triton-toluene base, permitted to darken for 48 hours, and then counted for 20 minutes in a liquid scintillation counting system. Appropriate quench corrections were made. Extracellular water space was calculated by comparing plasma specific activity to tissue specific activity.

Total tissue water was determined by heating additional mucosal samples to dryness. Total tissue water was taken as the difference between the wet and dry weights of the samples. Intracellular water was calculated by subtracting the individual values obtained for extracellular water from the mean value of total tissue water obtained from the same experiment.

Preliminary data indicate that acid peptic secreting mucosa exposed to topical acid contains $752 \pm 2 \mu\text{l}$ of total water, $239 \pm 14 \mu\text{l}$ of extracellular water, and, $509 \pm 14 \mu\text{l}$ of intracellular water/mgm of tissue. The influence of topical bile acid application on the same parameters is now being assessed.

V. PUBLICATIONS DURING THE CURRENT CONTRACT YEAR

Ritchie, W.P., Jr.: Bile acids, the "barrier" and reflux related clinical disorders of the gastric mucosa. Surgery 82:192-200, 1977.

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Ritchie, W.P., Jr., Nading, A., Shearburn, E.W., III: Relationship of transmural electrical potential difference to changes in gastric mucosal permeability to H^+ and blood flow. Am J. Surg. 135:110-114, 1978.

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